Leaf litter of some forest trees

Chemical composition and microbiological activity

ABSTRACT

Green and brown needles of Pinus sylvestris, *both on the tree and as litter after various lengths of time, were compared to determine differences in composition of various groups of lipophilic extractives, low-molecular-weight carbohydrates, cyclitols, phenolic glycosides, polysaccharides, lignin, ash, and crude protein. Also discussed is work in progress concerned with the manner in which extracts from these sources, as well as from spruce (*Picea abies*) and aspen (*Populus tremula*), affect some litter-decomposing and mycorrhizal fungi.*

KEYWORDS
Foliar analysis
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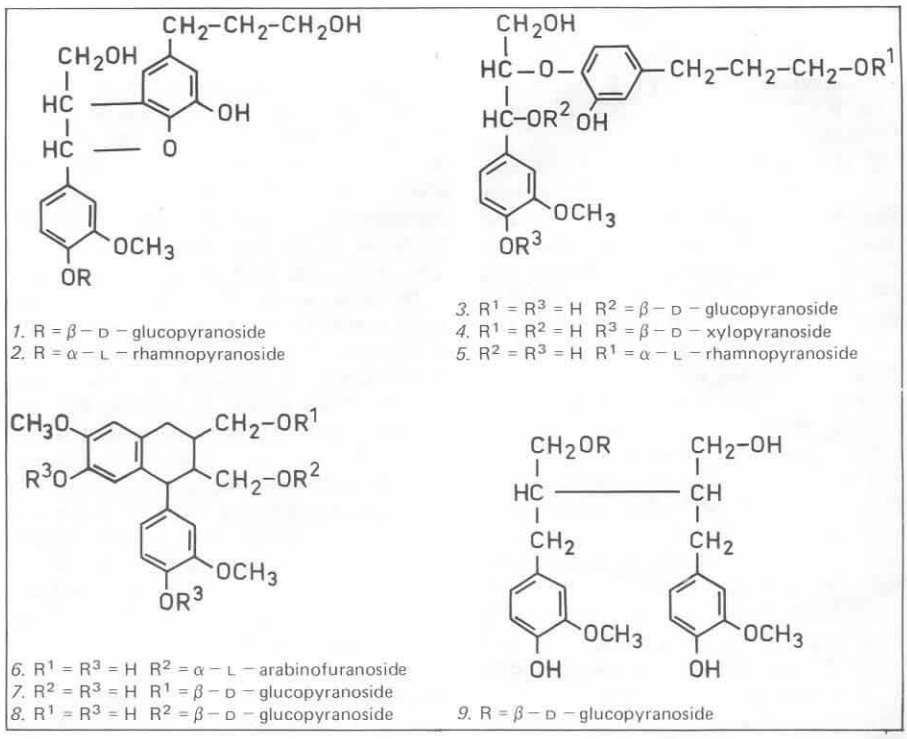
In connection with complete-tree utilization, knowledge of the chemical composition of foliage is most desirable. Since a high proportion of pine and spruce needles are brown (≈25%), it is of interest to compare the composition of these dead needles with that of the green ones. In particular, better understanding of the chemistry of dead needles and leaves and of their further decomposition in the litter stage is important in the study of problems concerning the accumulation, decomposition, and microbiological activity of organic matter in the soil.

In recent years, extensive studies of the lipophilic extractives of the so-called "technical" foliage from Scots pine (*Pinus sylvestris*) have been reported (*1*). The investigated samples consisted of 60–70% needles and 30–40% wood parts. The present paper discusses the differences in chemical composition in green and brown needles (on the tree as well as in litter form after various lengths of time) from Scots pine (*Pinus sylvestris*). Also discussed is work in progress on the chemistry of needles from Norway spruce (*Picea abies*) and preliminary studies on the


1. R = β – D – glucopyranoside
2. R = α – L – rhamnopyranoside
3. R¹ = R³ = H R² = β – D – glucopyranoside
4. R¹ = R² = H R³ = β – D – xylopyranoside
5. R² = R³ = H R¹ = α – L – rhamnopyranoside
6. R¹ = R³ = H R² = α – L – arabinofuranoside
7. R² = R³ = H R¹ = β – D – glucopyranoside
8. R¹ = R³ = H R² = β – D – glucopyranoside
9. R = β – D – glucopyranoside

1. *Dilignol glycosides isolated from green pine needles (*4, 5*).*

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effect on some forest fungi of extracts from these sources and from aspen leaves (*Populus tremula*).

Green needles

We have been interested in the constituents of conifer needles for many years (*2, 3*). Among the hydrophilic extractives (the water-soluble portion of an acetone extract) — mainly carbohydrates and the related cyclitols — compounds such as glucose, fructose, sucrose, pinitol, and shikimic acid were isolated in yields of 1.5–2.5% each (dry weight). Also present (0.1–1%) were arabinose, rhamnose, mannitol, melibiose, raffinose, myoinositol, and sequoitol, as well as traces of laminaribiose and cellobiose.

Also found in the hydrophilic portion of the acetone extract was a complex of phenolic glycosides and phenols. After separation of a group of monocyclic glycosides (of guaiacyl- and *p*-hydroxyphenylglycerol), we obtained from a Sephadex LH-20 column a complex mixture of dilignol glycosides, which has been fractionated and identified (*4, 5*) (Fig. 1), followed by dihydroquercetin-3′-β-glucoside (sometimes ≈2% of dry weight), quercetin-3′-β-glucoside, the corresponding aglycones, and (+)-catechin.

In the first group of glycosides, which are also present in other parts of the tree and are of interest in the biosynthesis of lignin, glucose is linked to hydroxyl groups in the glycerol side chain (*3*). Work on the elucidation of some of those compounds is still in progress. A summary of methods for the isolation and identification of the phenolic glycosides is given by Popoff and Theander (*4*). Of the dilignol glycosides, Compounds 1 (ca. 0.2%), 2 (ca. 0.4%), 3 (ca. 0.3%), and 4 (ca. 0.2%) of Fig. 1 predominate.

In the lipophilic portion of the acetone extract (soluble in methylene chloride), the main constituent (1–2%) consisted of pinifolic acid, a diterpene acid (*6*). A corresponding dehydro acid and benzoic acid were later identified among the other acid components (*7*).

The lipophilic extractives from spruce needles have not been studied so extensively as have been those from pine needles. However, there are some reports on phenolic compounds. Medvedeva *et al.* (*8*) reported a series of flavonoid glycosides and phenolic acids from Siberian spruces. We are at present studying the hydrophilic extractives from the needles of *Picea abies* (*9*). In addition to compounds previously found in spruces, Compounds 1, 2, and 3 (Fig. 1), which were previously found in pine needles (*4, 5*), have also been isolated. It is notable that we also found a glucoside corresponding to Compound 1 but with both phenolic groups methoxylated. Pinifolic and dehydropinifolic acids, previously found in pine needles (*6, 7*), were not found. Thompson *et al.* (*10*) have studied procyanidins (catechins) in *Pinus sylvestris* and *Picea abies*.

I. Extractives and residues from pine and spruce needles

	% (dry-weight basis)					
	Pine					Spruce
Extract	A	B	C	D	E	
Light petroleum	12.2	8.4	6.4	5.7	4.9	10.3
Ethyl acetate	5.0	6.5	9.7	9.8	9.4	3.1
2-Butanone	4.0	3.1	0.9	0.8	...	4.6
Water	19.9	9.2	11.6	12.5	7.9	27.1
Residue	65.4	73.2	74.4	79.6	83.0	58.7

II. Major lipophilic extractives from pine needles

	% (dry-weight basis)				
Group	A	B	C	D	E
Hydrocarbons	0.3	0.2	0.2	0.1	0.1
Steryl esters	1.2	0.6	0.5	0.3	0.2
Triglycerides	2.8	0.7	0.2	0.2	0.1
Free acids	1.1	1.2	1.0	1.2	1.0
Diterpene alcohols plus sterols	0.9	0.7	0.5	0.4	0.4

Brown needles

In other work, we have studied the chemical composition of brown needles from *Pinus sylvestris* and then followed chemical changes in these needles after different lengths of time on the forest floor. We are at present following the changes in that litter for a longer period of time than that discussed here (*11*).

In the tables in this paper, Sample A is a comparison sample of green pine needles collected several months prior to the gathering of the test samples. Sample B refers to the 4-year-old test sample picked from 12–15-year-old trees just before the falling season. Samples C, D, and E represent those portions of the test sample stored for 105, 165, and 358 days, respectively.

In the series A→E of Table I, one notices a steady decrease in the amount of the lipophilic (light petroleum) and *n*-butanone extractives as one progresses from green to brown, but an increase in the ethyl acetate extractives. Concentrations in the aqueous extractives drop considerably going from green to brown needles on the tree and then reach a maximum in the litter stage. There is an increase in the amount of residues from green to brown needles, and the amount of those residues is then further increasing.

The percentages of the major groups of compounds in the light petroleum extractives are given in Table II. Determination of the individual compounds in each group is in progress. Monoterpenes probably disappear during the analysis procedure being used, and polyisoprenols are not determined. There is a notable drop in the concentration of steryl esters and triglycerides from green to brown needles on the tree, followed by a further decrease in the litter. The amount of free acids is fairly constant, and hydrocarbon and alcohol concentrations drop slowly. Among the steryl esters, palmitic (16:0); oleic (9, 12–18:2); 5, 9, 12–18:3; and 9, 12, 15–18:3 acids are dominant constituents. In the triglycerides, these acids plus 9–18:1 acid predominate.

Among the free acids, the diterpene acids dominate, with pinifolic acid as the main component (ca. 0.6% of dry weight in all samples). β-Sitosterol and a diterpene alcohol of the labdane type are dominant components in the free sterol and alcohol mixtures, respectively.

In the ethyl acetate extracts are found aglycones of dilignol glycosides, dihydroquercetin, quercetin, and procyanidins (catechins). However, the number of identified compounds decreases going from green to brown needles, and further decreases are noted with increased time as litter. The number of more-condensed products, most of which are found in the ethyl acetate fraction, increases steadily. The phenolic glycosides are enriched in the *n*-butanone extract, and most of the low-molecular-weight carbohydrates and cyclitols are found in the aqueous extracts. The amounts of some of the identified compounds in these three extracts are given in Table III. Usually we find higher values of dihydroquercetin-3′-β-glucoside in needle samples than here in Sample A. There is a drastic drop in the amount of sugars and cyclitols going from green to brown needles. The small

III. Some hydrophilic extractives from pine needles

Compound	% (dry-weight basis) A	B	C	D	E
Glucose	2.3	0.4	0.3	0.2	0.1
Fructose	1.6	0.5	T[a]	...	...
Sucrose	0.7	0.2	T	...	...
Mannitol	1.8	0.1	0.2	0.1	0.1
Pinitol	2.2	0.4	0.1	0.1	T
Myoinositol	0.3	T	T	T	T
Glycoside 2	0.3	0.4	T	T	...
Dihydroquercetin-3′-β-glucoside	0.3	0.3	T	T	...

[a]Trace.

IV. Ash, crude protein, polysaccharides, and Klason lignin after extraction

	% (dry-weight basis) Pine A	B	C	D	E	Spruce
Ash	2.3	2.3	2.2	2.2	2.5	3.0
Crude protein	5.3	2.4	2.5	2.9	3.6	5.9
Acidic polysaccharide constituents	3.3	4.6	4.1	4.3	4.0	3.2
Neutral polysaccharide constitutents, calculated as:						
Glucans	25.5	19.9	23.7	29.4	27.4	16.3
Mannans	4.4	5.9	6.5	8.2	8.1	7.5
Galactans	1.8	2.8	3.1	3.1	2.9	1.5
Arabinans	2.6	4.0	3.1	2.6	2.3	3.1
Xylans	1.3	2.1	2.5	2.1	2.1	2.1
Klason lignin	14.8	22.6	25.7	28.4	31.3	14.4

amounts of glucose and mannitol found in the litter even after 1 year might result mainly from microbial polysaccharide degradation and/or synthesis.

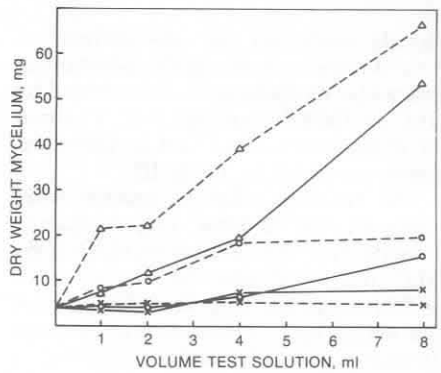
Concentrations of ash, crude protein (6.25 × N-value), polysaccharides (neutral and acidic), and Klason lignin in the residues after extraction were determined by conventional methods and are given in Table IV. The corresponding figures for needles from Norway spruce (*Picea abies*) are given for comparison. The ash concentrations are fairly stable, and there is a drop in the crude protein value going from green to brown needles. An increase with time in the crude protein value of the litter might indicate production of microbial protein and chitin. The amounts of polysaccharides in the different needle samples do not differ in a characteristic way, but the increasing content of Klason lignin with time shows a distinct trend. The lignin values also represent some nitrogen-containing compounds. Cellulose is clearly the predominating poysaccharide component, followed by mannose-containing hemicelluloses.

Fungi growth

Preliminary tests on the different types of extracts from Sample B indicate that the water extract exhibits a growth-promoting effect on the litter decomposers *Marasmius androsaceus* and *M. scorodonius*. The ethyl acetate extract showed a strong inhibiting effect on the latter fungus as well as on *Trichoderma polysporum* and *Cladosporum herbarum*. The mycorrhizal fungus *Boletus variegatus* was inhibited by the ethyl acetate extract, but somewhat stimulated by the aqueous extract, and the pine parasite fungus *Lophodermium pinastri* was strongly stimulated by the aqueous extract from the green needles (*12*).

The effect of the addition of different amounts of extract solutions from green spruce needles (containing concentrations of extracts in proportion to their contents in the needles) on the growth of the typical spruce-needle-decomposing fungus *Marasmius perforans* and the spruce parasite *Scleroderris lagerbergii* is illustrated in Fig. 2. A strong stimulating effect of the aqueous extract on the growth of both fungi is obvious. Also, some stimulation from the light petroleum extract is indicated.

Olsen *et al.* (*13*) have found that both green and yellow leaves of aspen (*Populus tremula*) contain a water-soluble compound that strongly stimulates the growth of litter-decomposing *Marasmius* fungi. Ethyl-acetate-soluble compounds (benzoic acid and catechol) strongly inhibited the growth of different mycorrhizal *Boletus* species, but inhibited less the growth of the *Marasmius* species.



2. Effect, compared with a glucose-containing control, of water (Δ), ethyl acetate (x), and light petroleum (o) extracts from spruce needles on the growth of Marasmius perforans *(– – –) and* Scleroderris lagerbergii *(—) (12).*

Experimental

Four-year-old needles (Sample B) were picked from 12–15-year-old trees at an experimental site at Jädraås, Sweden, in October just before the falling season. Samples C, D, and E were stored on the forest floor in bags of nylon net for 105, 165, and 358 days, respectively. The drop in dry weight of these three samples during storage was 10.4, 17.8, and 27.3%, respectively. The yields of various fractions and components given in the tables, however, are given on the basis of actual dry weight. As a comparison, a sample of green pine needles (Sample A) collected in May from the same trees was also studied. Some data from green needles of *Picea abies* collected in winter are also given as a comparison.

The samples were extracted for 2 × 0.5 hr with boiling acetone. After filtration, the needles were dried, milled, and extracted first with acetone (2 × 0.5 hr) and then with 50% aqueous acetone (2 × 0.5 hr) in an ultrasonic bath at room temperature. The combined extracts were evaporated to a small volume and by consecutive extractions divided into fractions comprising compounds soluble in light petroleum, ethyl acetate, 2-butanone (saturated with water), and water. The yields of extracts obtained by evaporation at reduced pressure (without further drying at elevated temperatures) are given in Table I. Also given are dry residues after extraction.

The light petroleum extractives were fractionated and the main groups of compounds (given in Table II) analyzed as previously described (*14*). The contents of phenolic glycosides and corresponding aglycones in the ethyl acetate, 2-butanone, and water extractives were determined after fractionation on Sephadex LH-20 columns as pre-

viously described (*5*). Low-molecular-weight carbohydrates in the 2-butanone and water extracts were determined by GLC as TMS derivatives (*15*). The major components identified in these extracts are given in Table III.

The residues after extraction were analyzed for neutral polysaccharide constituents (as alditol acetates) by GLC and for Klason lignin (*16*) after hydrolysis in sulfuric acid. The sum of the acidic polysaccharide constituents (uronic acids) was determined by a decarboxylation method (*17*), crude protein nitrogen by Kjeldahl analysis, dry matter by oven drying at 105°C for 18 hr, and ash content by drying at 600°C for 30 hr. Residue analyses are given in Table IV.

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